

RECEIVED
CENTRAL FAX CENTER

OCT 27 2003

OFFICIAL

Amendment

Patent Application
Docket no.: ACT-2001-UTL3

In the Claims

Please amend claims 82, 93, 95, 97, 110, and 112, and cancel claim 96, as follows:

1-81. (canceled)

82. (amended) A cascade genetic circuit comprising one or more nucleic acid constructs that comprise the components:

- a) a gene encoding a Nah-R transcriptional regulator; wherein said Nah-R transcriptional regulator is responsive to one or more benzoate derivatives;
- b) a PsaI promoter, wherein in the presence of at least one of said one or more benzoate derivatives, said PsaI promoter is activated by said Nah-R transcriptional regulator;
- c) a gene encoding the XylS2 transcriptional regulator, wherein said gene encoding the XylS2 transcriptional regulator is regulated by said PsaI promoter, and further wherein said XylS2 transcriptional regulator is responsive to at least one of said one or more benzoate derivatives; and
- d) a Pm promoter, wherein said Pm promoter is responsive to said XylS2 transcriptional regulator.

83. (previously presented) The cascade genetic circuit of claim 82, wherein said one or more nucleic acid constructs are provided in a prokaryotic cell.

84. (previously presented) The cascade genetic circuit of claim 82, wherein at least one of said one or more nucleic acid constructs is provided in a plasmid that can replicate in gram negative bacteria.

85. (previously presented) The cascade genetic circuit of claim 84, wherein at least one of said one or more nucleic acid constructs are provided in at least one plasmid that can

promote the integration of at least one of said components of said cascade genetic circuit into the chromosome of said gram negative bacteria.

86. (previously presented) The cascade genetic circuit of claim 82, wherein at least one of said one or more nucleic acid constructs is integrated into the chromosome of a gram negative bacteria.
87. (previously presented) The cascade genetic circuit of claim 82, wherein said one or more nucleic acid constructs are two or more nucleic acid constructs.
88. (previously presented) The cascade genetic circuit of claim 87, wherein said two or more nucleic acid constructs are two nucleic acid constructs.
89. (previously presented) The cascade genetic circuit of claim 88, wherein said two nucleic acid constructs comprise:
 - a) a first nucleic acid construct that comprises a regulatory cassette comprising said gene encoding a Nah-R transcriptional regulator, said PsaI promoter, and said gene encoding said XylS2 transcriptional regulator, wherein said gene encoding said XylS2 transcriptional regulator is regulated by said PsaI promoter; and
 - b) a second nucleic acid construct that comprises an expression cassette comprising said Pm promoter.
90. (previously presented) The cascade genetic circuit of claim 89, wherein said Pm promoter of said expression cassette is positioned upstream of and in proximity to one or more restriction sites, wherein the expression of a nucleic acid sequence cloned into at least one of said one or more restriction sites can be regulated by said Pm promoter.
91. (previously presented) A prokaryotic cell comprising the cascade genetic circuit of claim 82.

92. The prokaryotic cell of claim 91, wherein said prokaryotic cell is a gram negative bacterial cell.
93. (amended) A cascade genetic circuit comprising one or more nucleic acid constructs that comprise the components:
- a) a gene encoding a Nah-R transcriptional regulator; wherein said Nah-R transcriptional regulator is responsive to one or more benzoate derivatives;
 - b) a P_{sal} promoter, wherein in the presence of at least one of said one or more benzoate derivatives, said P_{sal} promoter is activated by said Nah-R transcriptional regulator;
 - c) a gene encoding the XylS2 transcriptional regulator, wherein said gene encoding the XylS2 transcriptional regulator is regulated by said P_{sal} promoter, and further wherein said XylS2 transcriptional regulator is responsive to at least one ~~or~~ of said one or more benzoate derivatives;
 - d) a P_m promoter; wherein said P_m promoter is responsive to said XylS2 transcriptional regulator; and
 - e) a nucleic acid sequence in proximity to said P_m promoter, wherein the expression of said nucleic acid sequence is regulated by said P_m promoter.
94. (previously presented) The cascade genetic circuit of claim 93, wherein said nucleic acid sequence encodes a peptide.
95. (amended) The cascade genetic circuit of claim 93, wherein said nucleic acid sequence encodes an antisense molecule, a ribozyme, an rRNA, a tRNA, an snRNA, or a diagnostic RNA molecule.
96. (canceled) The cascade genetic circuit of claim 93, wherein said Nah-R transcriptional regulator is a mutant form of Nah-R.

Patent Application
Docket no.: ACT-2001-UTL3

97. (amended) The cascade genetic circuit of claim 9693, wherein said Nah-R transcriptional regulator is a mutant form of Nah-R is selected from the group consisting of nahR3 or and nahR4.
98. (previously presented) A prokaryotic cell comprising the cascade genetic circuit of claim 93.
99. (previously presented) The prokaryotic cell of claim 98, wherein said prokaryotic cell is a gram negative bacterial cell.
100. (previously presented) The cascade genetic circuit of claim 93, wherein at least one of said one or more nucleic acid constructs is provided in a plasmid that can replicate in gram negative bacteria.
101. (previously presented) The cascade genetic circuit of claim 100, wherein at least one of said one or more nucleic acid constructs are provided in at least one plasmid that can promote the integration of at least one of said components of said cascade genetic circuit into the chromosome of said gram negative bacteria.
102. (previously presented) The cascade genetic circuit of claim 93, wherein at least one of said one or more nucleic acid constructs is integrated into the chromosome of a gram negative bacteria.
103. (previously presented) The cascade genetic circuit of claim 93, wherein said one or more nucleic acid constructs are two or more nucleic acid constructs.
104. (previously presented) The cascade genetic circuit of claim 103, wherein said two or more nucleic acid constructs are two nucleic acid constructs.

105. (previously presented) The cascade genetic circuit of claim 104, wherein said two nucleic acid constructs comprise:
- a) a first nucleic acid construct that comprises a regulatory cassette comprising said gene encoding a Nah-R transcriptional regulator, said PsaI promoter, and said gene encoding said XylS2 transcriptional regulator, wherein said gene encoding said XylS2 transcriptional regulator is regulated by said PsaI promoter; and
 - b) a second nucleic acid construct that comprises an expression cassette comprising said Pm promoter and said nucleic acid sequence in proximity to said Pm promoter.
106. (previously presented) A prokaryotic cell comprising the cascade genetic circuit of claim 105.
107. (previously presented) The prokaryotic cell of claim 106, wherein said prokaryotic cell is a gram negative bacterial cell.
108. (previously presented) The prokaryotic cell of claim 107, wherein said prokaryotic cell is an E. coli cell.
109. (previously presented) The prokaryotic cell of claim 107, wherein said prokaryotic cell is a Pseudomonas putida cell.
110. (amended) A method of inducing the expression of a nucleic acid sequence, comprising:
- a) providing or establishing the cascade genetic circuit of claim 93 in a gram negative bacteria; and
 - b) contacting said gram negative bacteria with at least one benzoate derivative to induce the expression of said nucleic acid sequence.

Patent Application
Docket no.: ACT-2001-UTL3

111. (previously presented) The method of claim 110, wherein said nucleic acid sequence encodes a polypeptide.
112. (amended) The cascade genetic circuit of claim 93, wherein said nucleic acid sequence encodes an antisense molecule, a ribozyme, an rRNA, a tRNA, an snRNA, or a diagnostic RNA molecule.
113. (previously presented) The method of claim 110, wherein said benzoate derivative is salicylate or a substituted salicylate molecule.
114. (previously presented) The method of claim 110, wherein said gram negative bacteria is *E. coli*.
115. (previously presented) The method of claim 110, wherein said gram negative bacteria is *Pseudomonas putida*.